

Functional and Phenotypic Comparison of Human T Cell Leukemia/Lymphoma Virus Positive Adult T Cell Leukemia with Human T Cell Leukemia/Lymphoma Virus Negative Sézary Leukemia, and Their Distinction Using Anti-Tac Monoclonal Antibody Identifying the Human Receptor for T Cell Growth Factor

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Abstract. Adult T cell leukemia (ATL) and Sézary leukemia are malignant proliferations of T lymphocytes that share similar cell morphology and clinical features. ATL is associated with HTLV (human T cell leukemia/lymphoma virus), a unique human type C retrovirus, whereas most patients with the Sézary syndrome do not have antibodies to this virus. Leukemic cells of both groups were of the T3, T4-positive, T8-negative phenotype. Despite the similar phenotype, HTLV-negative Sézary leukemic cells frequently functioned as helper cells, whereas some HTLV-positive ATL and HTLV-positive Sézary cells appeared to function as suppressors of immunoglobulin synthesis. One can distinguish the HTLV-positive from the HTLV-negative leukemias using a monoclonal antibody (anti-Tac) that appears to identify the human receptor for T cell growth factor (TCGF). Resting normal T cells and most HTLV-negative Sézary cells were Tac-negative, whereas all ATL cell populations were Tac-positive. The observation that ATL cells manifest TCGF receptors suggests the possibility that an abnormality of the TCGF-TCGF receptor system may partially explain the uncontrolled growth of these cells.

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Introduction

In normal individuals, circulating T cells represent a complex mixture of cells with different and at times opposing functions. Leukemic T cells from humans frequently represent expansions of a single clone of T cells that may retain a single immunoregulatory function. The examination of such neoplastic T cells and their soluble products in functional assays has provided valuable insights into the regulatory network of cells that control the human immune response in general and control B cell maturation and immunoglobulin production in particular (1-3). Furthermore, the use of monoclonal antibodies to T cell surface antigens has permitted useful correlations between cell function and phenotype (4-9).

In the present studies we examined leukemias of mature T cells. The first form, the Sézary syndrome, is characterized by exfoliative erythroderma, generalized lymphadenopathy, and circulating pleomorphic malignant T lymphocytes that have a propensity for epidermal infiltration. The second leukemia, the adult T cell leukemia (ATL)¹, was recently described by Uchiyama et al. (10). ATL is also a malignancy of mature T cells that has a tendency to infiltrate the skin. However, certain clinical features aid in distinguishing these syndromes. First, ATL has a more aggressive course and is often complicated by hypercalcemia and pulmonary infiltrates. The median survival is generally 1 yr or less (10, 11). Second, cases of ATL are clustered geographically, occurring in the southwest of Japan, the Carib-

1. Abbreviations used in this paper: ATL, adult T cell leukemia; FACS, fluorescence-activated cell sorter; HLA, human leukocyte antigen; HTLV, human T cell leukemia/lymphoma virus; PWM, pokeweed mitogen; TCGF, T cell growth factor.

bean basin, and in certain areas of the southeastern U. S. (11, 12). Recently, a unique human type C retrovirus, human T cell leukemia/lymphoma virus (HTLV), has been isolated from the neoplastic T cells of patients with ATL in multiple areas of the world (13–19). This virus was distinguished from other mammalian retroviruses by assays of nucleic acid homology and immunological assays of viral proteins. In a wide survey of human sera for antibodies to HTLV, nearly all serum samples from patients with ATL contained antibodies recognizing the core p19 and p24 proteins of this virus (20, 21). Furthermore, up to 15% of normal donors in endemic areas of Japan and the Caribbean basin had antibodies to these core proteins. In contrast, <1% of normal donors from nonendemic areas of the world have demonstrable antibodies to HTLV. Similarly, only 2 of 245 (0.8%) patients with the Sézary syndrome, or mycosis fungoides, had serum antibodies recognizing HTLV (22).

In the present study, we compare the immunoregulatory function and cell surface phenotype of the leukemic cells from 10 patients with the clinical diagnosis of ATL and three patients with the Sézary leukemia who had circulating antibodies to HTLV, with the function and cell surface phenotype of leukemic cells from 10 patients with the Sézary syndrome who lacked serum antibodies to HTLV.

Methods

Patient population. T cells were studied from 17 normal individuals, 10 patients with the Sézary syndrome who did not have serum antibodies to HTLV, three patients with the Sézary syndrome who had serum antibodies to HTLV, and 10 patients with the ATL. The patients with the clinical presentation of the Sézary syndrome had exfoliative erythroderma, lymphadenopathy, and a white blood cell count ranging from 12,600 to 124,000 cells/mm³. With the exception of the one patient with a total white blood cell count of 12,600, all patients had >30,000 white blood cells/mm³. All of the patients were from the U. S. and had a mean age of 62. Nine of the 10 patients without antibodies to HTLV were white, while one was black. The patients with ATL had skin involvement, usually in the form of plaquelike lesions, and had white blood cell counts that ranged from 11,200 to 105,000/mm³. Three of these patients had a white blood cell count of between 11,000 and 20,000/mm³. Six of the patients were hypercalcemic. Three of the patients were Oriental (residing in Japan), eight were black, and one was white. One of these latter nine patients was from the Caribbean while the remainder resided in the U. S. The mean age of this patient group was 39. In certain cases, the classification of patients on the basis of clinical features was difficult. In our analyses, patients with antibodies to HTLV were compared with those without antibodies to HTLV; this was done irrespectively of the initial clinical diagnosis.

Separation of T and B cells. Mononuclear cells were separated from peripheral blood by Ficoll-Hypaque density gradient centrifugation. T and B cells were prepared from peripheral blood mononuclear cells by a combination of Sephadex G-200 anti-human F(ab')₂ immunoabsorbent chromatography and rosette formation with neuraminidase-treated sheep red blood cells, as previously described (1).

Preparation of leukemic cells for study. Mononuclear cells, including

the leukemic cells from patients with either T cell leukemia, were purified from heparinized venous blood by Ficoll-Hypaque gradient centrifugation. After thorough washing and resuspension in RPMI 1640 culture media (Gibco Laboratories, Grand Island, NY) and 10% fetal calf serum, these cells were studied for their capacity to synthesize immunoglobulin molecules or to function either as helper or suppressor cells in *in vitro* assays of pokeweed mitogen (PWM)-activated immunoglobulin production.

Production and reactivities of monoclonal antibodies. The OKT3, T4, T6, T8, T9, and T10 antibodies were obtained from Ortho Pharmaceuticals, Raritan, NJ. DA2 (a kind gift from Drs. Peter Param and Jack Strominger, Harvard Medical School, Boston, MA) is a monoclonal antibody directed toward a non-polymorphic framework determinant of human Ia-like antigens (23). We also determined the reactivity of the leukemic cell populations with a monoclonal antibody of the IgG2a class termed anti-Tac prepared in our laboratory (8, 24) that reacts with the human receptor for T cell growth factor (TCGF) (9, 25).

Immunofluorescence analysis of cells with the fluorescence-activated cell sorter (FACS). The cells were analyzed by using immunofluorescence and FACS analysis with monoclonal antibodies by a slight modification of a procedure described previously (26). Briefly, 1×10^6 cells were incubated with saturating amounts of monoclonal antibodies or 0.05 ml of BALB/c normal mouse serum for 30 min at 4°C in Hanks balanced salt solution, which contained 0.05% sodium azide and 3% fetal calf serum (Armour Pharmaceutical Co., Scottsdale, AZ). The cells were then washed twice in this media and reacted with saturating amounts of fluorescein isothiocyanate-goat F(ab')₂ anti-mouse IgG for 30 min at 4°C, washed twice, and resuspended for analysis. Analysis was performed using a B-D FACS II (Becton-Dickinson & Co. FACS Systems, Sunnyvale, CA) that was interfaced to a PDP11/34 computer (Digital Equipment Corp., Maynard, MA). Data were collected on $2-5 \times 10^4$ viable cells (as determined by forward light scatter intensity) as fluorescence intensity vs. cell number. Histograms for each cell type were integrated to determine the percentage of positive cells when reacted with individual monoclonal antibodies relative to a negative control of cells reacted with normal mouse serum.

Assays for helper or suppressor cell activity. The methods used to test for immunoglobulin biosynthesis and for helper and suppressor activity using the PWM-driven immunoglobulin biosynthesis system have been described in detail previously (1, 2, 27, 28). PWM-stimulated B cells in the absence of T cells produce very small amounts of immunoglobulin (1, 28, 29). The addition of autologous or allogeneic T cells will lead to an enhancement of immunoglobulin production by these rigorously T cell-depleted B cells. Thus, the ability of added leukemic T cells to enhance immunoglobulin production by normal B cells can be used as an index of helper cell function. Suppressor cell activity was assessed by determining the amount of immunoglobulin produced and secreted into the media by the PWM-stimulated co-cultures of leukemic cells and by indicator cells consisting of mixtures of equal numbers of normal B cells and normal irradiated (2,000 rad) T cells. Irradiated T cells were shown to maintain helper T cell activity but could not be activated to suppress in these co-cultures. Ratios of 5×10^5 leukemic T cells to 5×10^5 normal irradiated T cells and 5×10^5 normal B cells were used in these co-culture studies. The immunoglobulin synthesis in these co-cultures was compared with that predicted from the determinations of the immunoglobulin synthesis by the PWM-stimulated indicator population and leukemic cell populations when cultured alone.

$$\% \text{ suppression} = 1 - \frac{\text{ng of Ig secreted by the PWM-stimulated co-culture of leukemic cells with indicator B and irradiated T cells}}{\text{sum of ng of Ig secreted by indicator PWM-stimulated B and irradiated T cells and of the leukemia cell population when cultured alone}} \times 100$$

The amount of immunoglobulin produced and secreted into the media after 12 d in culture in the biosynthesis studies discussed above was determined by heavy chain specific double antibody radioimmunoassays, as previously described (27).

Assays for antibodies to HTLV. The sera of patients with the Sézary syndrome and ATL in the present study were analyzed for antibodies to the core proteins p19 and p24 of HTLV using solid-phase radioimmunoassays, as previously described (20, 21).

Results

The capacity of peripheral blood mononuclear cells from patients with the Sézary syndrome, ATL, and normal individuals to synthesize IgG, IgA, and IgM was evaluated using the *in vitro* PWM-stimulated immunoglobulin biosynthesis system. The 17 normals studied synthesized geometric means of 3,147 ng (2.9)² for IgG, 2,289 ng (2.54) for IgA, and 3,676 ng (2.57) for IgM per two million cells in culture. In contrast, none of the peripheral blood mononuclear cell populations from the patients with the Sézary syndrome or the ATL synthesized biologically meaningful quantities of immunoglobulin of any class when stimulated by PWM (Fig. 1). The PWM-activated *in vitro* biosynthesis system was also used to determine whether the neoplastic T cells manifested helper activity when co-cultured with rigorously T cell-depleted B cells from normal individuals. The approach takes advantage of the fact that PWM stimulation of immunoglobulin synthesis has an absolute requirement for helper T cells (1, 28, 29). When normal B cells were co-cultured with either autologous or allogeneic normal T cells and PWM, they matured into immunoglobulin synthesizing and secreting cells (Fig. 1). In the present study, purified PWM-stimulated, normal B cells did not synthesize immunoglobulin molecules when cultured alone. However, they synthesized and secreted from 250 to over 5,000 ng of IgM when cultured with Sézary leukemic T cells of six of the nine patients studied, at a ratio of 5×10^4 Sézary cells to 5×10^5 normal B cells in culture (Fig. 1). The synthesis of IgG and IgA in these co-cultures was comparable (data not shown). Thus, the leukemic cells of many but not all of the patients with the anti-HTLV antibody-negative Sézary syndrome functioned as helper T cells for normal B cell maturation and immunoglobulin synthesis. In contrast, the ATL cells and the leukemic cells from the three Sézary syndrome patients who had antibodies to HTLV did not manifest helper cell activity for B cell maturation and immunoglobulin synthesis (Fig. 1).

In order to determine whether the leukemic cell populations had suppressor cell activity, circulating mononuclear cells from the patients were cultured with PWM-stimulated indicator populations comprised of unseparated peripheral blood mononuclear cells from normal subjects or equal numbers of normal B cells and normal irradiated T cells (2,000 rad). In control cultures, the mean values for the production of IgG, IgA, and IgM by 10 co-cultures of allogeneic lymphocytes from pairs of normal individuals ranged from 95 to 106% of the expected values. None of the leukemic cell populations from any of the

patients with HTLV antibody-negative Sézary leukemia studied manifested suppressor cell activity (Fig. 1). In contrast, the leukemic cells of five of the nine patients with ATL suppressed immunoglobulin synthesis when co-cultured with indicator populations of equal numbers of normal B cells and irradiated normal T cells (Fig. 1). In addition, the Sézary leukemic cells of the three patients with antibodies to HTLV suppressed the IgM synthesis of co-cultured PWM-activated normal indicator cells by 12, 52, and 54%, suppressed IgG synthesis by 54, 46, and 58%, and suppressed IgA synthesis by 36, 45, and 52%. The leukemic cells from this group of patients with antibodies to HTLV also suppressed immunoglobulin synthesis when cultured with normal indicator unseparated peripheral blood mononuclear cells (data not shown). In the one case of ATL examined, the leukemic cells inhibited the immunoglobulin synthesis of B cells and co-cultured normal T cells that had been depleted of T8-positive cells by prior treatment with anti-T8 monoclonal antibody and complement. These latter T cells were shown to contain <1% of T8-positive cells by FACS analysis (Becton-Dickinson & Co. FACS Systems). Thus, in contrast to the helper immunoregulatory function of leukemic cells that was demonstrable for certain patients with anti-HTLV antibody-

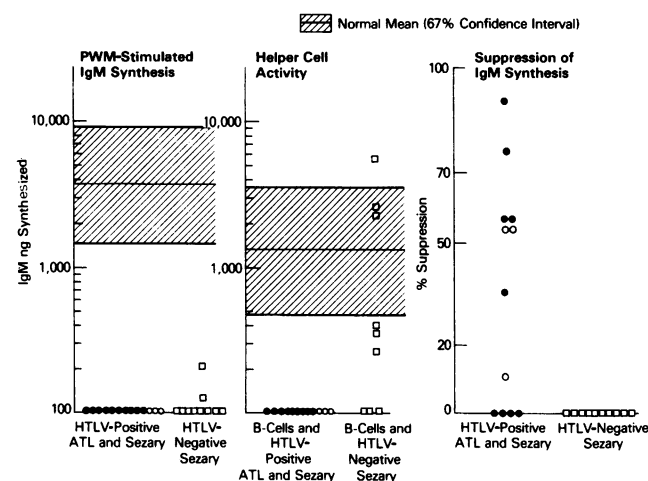


Figure 1. Immunoregulatory functions of HTLV-positive Sézary (○) and ATL cells (●) and of HTLV-negative Sézary leukemic T cells (□). As indicated in the panel on the left, there was no biologically meaningful IgM synthesis by PWM-stimulated ATL or Sézary leukemic T cells. The geometric mean and 67% confidence interval for immunoglobulin synthesis by 17 normal individuals are indicated by the central line and crosshatched area, respectively. None of the HTLV-positive ATL and Sézary leukemic cells provided helper function for co-cultured normal B cells (center). In contrast, there was from 250 to over 5,000 ng of IgM synthesized by 5×10^5 normal B cells when co-cultured with 5×10^4 HTLV-negative Sézary leukemia T cells, which indicated that these latter cells manifested helper activity for B cells. As indicated in the right panel, 8 of the 12 HTLV-associated leukemic (nine ATL indicated by ●, three Sézary by ○) populations examined suppressed the IgM synthesis of co-cultured normal B cells and irradiated T cells. None of the HTLV-negative Sézary T cell populations examined suppressed immunoglobulin synthesis.

2. The geometric means are presented with 1 SD of the log of the geometric mean given in parentheses.

negative Sézary syndrome in these in vitro cultures, the leukemic cells of some patients with HTLV-associated leukemias, including ATL patients and the three Sézary patients with antibodies to HTLV, appear to function as suppressor cells.

Over the past few years there have been major advances in the correlation of the cell surface antigens of leukemic T cells identified by monoclonal antibodies with the immunoregulatory function of these cells (4-6). However, the T3, T4, and T8 monoclonal antibodies were not of value in differentiating HTLV-negative Sézary leukemic cells, which appear to manifest helper activity from either ATL cells or the Sézary leukemic cells from the three patients with serum antibodies to HTLV that appear to manifest suppressor activity. In general, both of these groups of leukemic cells bore the T3 antigen associated with mature T cells and the T4 antigen that has usually been associated with helper/induced T cell activity, whereas neither of the leukemic cell populations manifested the T8 antigen that is usually associated with suppressor/cytotoxic activity (Fig. 2).

We then examined these leukemic cell populations using monoclonal antibodies to a series of activation antigens, including the T9 antigen, which defines the transferrin receptor (7), the Ia antigen (using the DA2 monoclonal), present on resting B cells, monocytes, and on activated T cells, the T10 antigen, which is present on primitive hemopoietic cells, thymocytes, NK cells, and on activated T cells (30), and the Tac antigen, an antigen that recognizes the TCGF receptor on activated T cells (8). Both T9 and Ia antigen were present on the leukemic cells of the majority of patients with either the HTLV-positive ATL or the Sézary T cell leukemias, that had no serum antibodies to HTLV, but were not present on normal resting

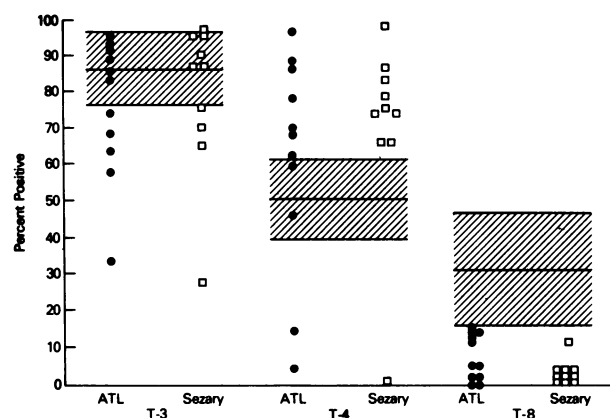


Figure 2. Cell surface phenotypes of ATL (●) and HTLV antibody negative Sézary leukemic cells (□). The majority of the leukemic cell populations of both leukemic groups were positive when analyzed with the T3 and T4 monoclonal antibodies, but were negative when analyzed with the T8 monoclonal. The mean value ± 2 SD for the proportion of T cells reactive with these monoclonal antibodies for 10 normal individuals is indicated by the line and crosshatched area, respectively.

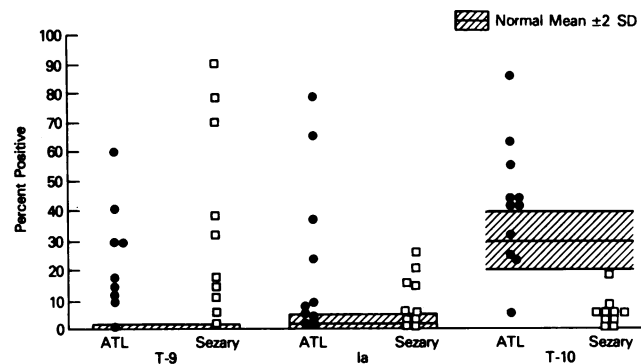


Figure 3. The reactivity of ATL (●) and Sézary leukemia (□) cells with T9, Ia, and T10 monoclonal antibodies. The majority of ATL and Sézary leukemic cells were T9- (transferrin receptor) positive. In addition, a number of leukemic populations from both types of leukemias were Ia-positive. 10 of 11 patients with HTLV-associated leukemias studied had normal or elevated proportions of T10-positive cells. In contrast, all of the 10 Sézary leukemic populations had a lower proportion of cells reactive with the T10 monoclonal than did normal individuals.

T cells (Fig. 3). Thus, these monoclonal antibodies help to identify both of these groups of leukemic cells as activated, rapidly proliferating cells, but they are not of value in differentiating these leukemias from each other. The monoclonal antibody T10 was of greater value in differentiating these leukemias. The 10 HTLV antibody negative Sézary leukemic cell populations had reduced proportions of T10 cells, whereas 10 of 11 HTLV-positive ATL or Sézary leukemic populations examined had normal or elevated proportions of T10-positive T cells.

The monoclonal antibody, anti-Tac, which was developed in our laboratory, appears to react with the membrane receptor for TCGF (8, 9, 25). The anti-Tac monoclonal was of value in differentiating cells from the patients with ATL and from the three Sézary patients with antibodies to HTLV from the cells of the remaining Sézary patients, who were anti-HTLV antibody-negative. Nine of the ten Sézary leukemic T cell populations from patients that did not have antibodies to HTLV were Tac antigen-negative, whereas all of the leukemic T cell populations from patients with antibodies to HTLV were Tac antigen-positive (Fig. 4). Thus, the HTLV-associated mature T leukemia cells were Tac antigen-positive, and thus presumably manifest the receptor for TCGF. This conclusion has been verified by showing that these same cells proliferate in the presence of TCGF.

Discussion

The Sézary T cell leukemia and the ATL share several characteristics. In individual cases it may not be possible to make a distinction on clinical grounds alone. Both are leukemias of mature pleomorphic T cells with a propensity to invade the skin. Both affect adult individuals. Furthermore, the leukemic

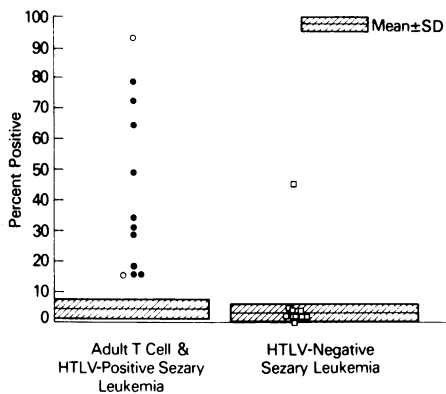


Figure 4. Reactivity of ATL and HTLV-negative Sézary leukemic cells with anti-Tac (anti-TCGF receptor antibody). All of the ATL cell populations (●) as well as the three HTLV-positive Sézary leukemic cell populations (○) contained cells that were reactive with the anti-Tac monoclonal antibody. In contrast, 9 of the 10 Sézary leukemic populations (□) from patients who did not have antibodies to HTLV were Tac antigen-negative.

cells from both groups of patients bear a similar surface phenotype when analyzed with T3, T4, and T8 monoclonal antibodies. There are, however, certain characteristics which, when present (e.g., a tendency for the ATL patients to develop hypercalcemia), aid in distinguishing these leukemias from each other (10–12). ATL has a definite geographical clustering, and virtually all patients have serum antibodies to HTLV. HTLV has been isolated from the leukemic cells of such patients (22). In contrast, only 2 of 245 patients with the cutaneous T cell lymphomas, including mycosis fungoides and the Sézary syndrome, had circulating antibodies to the p19 and p24 core proteins of HTLV (22). In the present study, three patients with antibodies to HTLV carried the diagnosis of Sézary syndrome on the basis of clinical features, and HTLV was isolated from their leukemic cells (18). These patients were included with the ATL patients in our analysis, since the Sézary leukemia cells from patients with antibodies to HTLV manifested suppressor function and the Tac-positive phenotype, thus presenting a similar picture to that of the HTLV-associated ATL patients.

There were major differences demonstrable between the HTLV antibody positive T cell leukemias and the HTLV antibody-negative Sézary T cell populations when their immunoregulatory function was assessed using an *in vitro* immunoglobulin biosynthesis system. In the present study, six of the nine HTLV antibody-negative Sézary T cell leukemic populations function as helper cells when co-cultured with normal B cells in the presence of PWM. This observation is in accord with our previous report that some, but not all, Sézary T cell leukemic populations are dedicated to helper interactions with B lymphocytes, which facilitates B cell proliferation, maturation, and immunoglobulin synthesis (1). Our initial observation has been confirmed by other workers (31) studying similar cell pop-

ulations, although two cases of a Sézary leukemia functioning as a suppressor cell have been reported (32, 33). These latter cases were reported before the discovery of either ATL or HTLV.

The ATL cells studied contrast with the HTLV-negative Sézary leukemic cells, in that they did not manifest helper activity in any case. However, in 6 of the 10 cases of ATL studied and in the three cases of HTLV-associated Sézary syndrome, the leukemic cells functioned as suppressor cells and inhibited the immunoglobulin synthesis of co-cultured, PWM-stimulated, normal mononuclear target cell populations. This observation is in accord with previous studies with this form of leukemia (34). Such an apparent suppressor activity could occur *in vitro* through a number of mechanisms. First, leukemic T cells with receptors for TCGF have the potential to absorb TCGF from the media, leading to inhibition of T cell-dependent immunoglobulin synthesis. Second, the leukemic T cells could theoretically act as inducer T cells and activate normal precursors of suppressor cells into suppressor effectors. Third, HTLV could conceivably act on B cells directly to inhibit their function. Finally, the leukemic T cells could function as effectors of suppression. In terms of the first alternative, we have added 250 ng of purified TCGF to the co-cultures of 10^6 leukemic and 10^6 indicator cells without affecting the 75% inhibition of immunoglobulin synthesis observed in these co-cultures. Since this quantity of TCGF is 10- to 100-fold greater than that required to saturate the TCGF receptors on the ATL cells, it is unlikely that absorption of TCGF is the cause of the apparent suppression. In terms of the second alternative, in the present study, the leukemic T cells from individuals with a suppressor leukemia inhibited target B cells and irradiated T cells or target B cells and T8 cell-depleted T cell populations. These observations make less likely the possibility that the leukemic T cells induce a cell among the normal T cells to become the effectors of suppression. It is still possible that the leukemic cells induced T8-positive cells, contaminating the leukemic cells to become the effectors of suppression. We have made one experimental observation that argues against this alternative as well as against the possibility that leukemic cells absorb TCGF. We have established a long-term TCGF-dependent line from the Tac-positive leukemic cells of one of the patients with an HTLV-associated leukemia. This line was shown to secrete a 70–90,000-mol wt suppressor protein termed SISS-B resistant to RNase and DNase treatment that inhibits PWM-induced immunoglobulin synthesis by B cells and irradiated T cells as well as the immunoglobulin synthesis induced by Epstein-Barr virus in T cell-depleted short-term B cell cultures (35). This suppressor molecule does not inhibit T cell proliferation. The suppressor T cell line has the surface phenotype T3+, T4+, Tac+, Ia+, and T8 – 3AI–, and contains HTLV DNA integrated sequences (13) similar to the leukemic cells from this patient. The third alternative, that HTLV from ATL cells inhibited B cell immunoglobulin synthesis, also appears unlikely, since irradiation of the ATL cells abrogates their capacity to suppress immunoglobulin synthesis but not their ability to transform co-cultured

activated cord blood T cells. Furthermore, doubly banded purified HTLV did not inhibit immunoglobulin synthesis, whereas a culture supernatant from a transformed cord T cell line that was HTLV nonproducing inhibited immunoglobulin synthesis in the *in vitro* co-culture system. Thus, in contrast to the helper immunoregulatory function of T3+T4+T8- leukemic T cells from some patients with the Sézary syndrome, certain patients with ATL with the same phenotype have malignant T cells that appear to be cells of the suppressor T cell network. It appears from these studies, as well as from more extensive analyses from other laboratories (36-38), that the cells defined by the T4 and T8 antibodies are complex populations that are not solely helper/inducer and suppressor/cytotoxic populations, respectively. The studies from other laboratories (36-38) concerning cytotoxic lymphocytes support the view that cell populations that react with T4 and T8 monoclonal antibodies differ not in terms of their function *per se*, but in terms of the class of histocompatibility antigen recognized by the T cell; that is, T8-positive cells appear to be involved in those cellular interactions and functions that involve recognition of class I human leukocyte antigen (HLA) molecules (i.e., HLA A or B molecules), whereas T4-positive cells are involved in interactions that involve recognition of class II molecules (i.e., HLA-D or SB). In this scheme, T4-positive cells would not only be helper cells in their interactions with other lymphoid cells but might also act as cytotoxic cells when HLA-D molecules are involved. In addition, Thomas et al. (39) have shown that T4-positive cells can function as effectors of suppression when no T8-positive cells are present in the culture system. Thus, at least for the antigen nonspecific PWM system examined, certain normal T4-positive cells as well as certain T4-positive ATL cell populations may function as suppressors in the PWM-induced immunoglobulin biosynthesis system.

Recently, the proliferation and maturation of T cells has been shown to be regulated by a series of growth factors and their receptors. Stimulation of unseparated mononuclear cells leads to the production of interleukin 1 by monocytes. Following interaction with interleukin 1 and the primary stimulatory lectin or antigen, T cells become activated and secrete TCGF (40). TCGF interacts with an inducible receptor that is present on activated peripheral blood T cells and TCGF-dependent continuous T cell lines, but is not present on resting T cells, B cells, or monocytes. We have prepared a monoclonal antibody termed anti-Tac that recognizes the receptor for TCGF (8, 9, 25).

Anti-Tac appears to be of considerable value in differentiating the HTLV-associated T cell leukemic cells from the malignant cells of those patients who have a leukemia of mature T cells but do not have antibodies to HTLV. The T cell leukemic cell populations from 9 of the 10 patients with the Sézary syndrome who were HTLV antibody-negative, were Tac antigen-negative. All nine ATL populations and the T cell leukemic populations from the three Sézary patients that had circulating antibodies to HTLV were Tac antigen positive. Concordance between serum antibodies to HTLV and Tac-positive leukemic T cells

should not be exact. For example, in the HTLV endemic regions of Japan, the Caribbean, and southeastern U. S. where from 3 to 25% of the population may have antibodies to HTLV (41), one might expect to find patients with Tac-negative leukemias and other cancers associated with antibodies to HTLV by chance. In such cases it would be important to determine whether the Tac-negative leukemic T cells bore the HTLV by molecular hybridization studies. Furthermore, we observed a Tac-positive leukemic cell in a patient with Sézary leukemia lacking antibodies to HTLV core proteins. This could occur by a number of mechanisms: (a) the patient's leukemic cells could be infected with a defective (i.e., nonproductive) form of HTLV; (b) the leukemia could be caused by a retrovirus of the HTLV family but not of the class (subgroup) of HTLV (HTLV-I) from which the test proteins are available for these assays; or (c) these cells could be activated to manifest this receptor by other unidentified mechanisms.

The ATL cells or leukemia T cell lines associated with a productive form of HTLV we have studied have been Tac-positive. A number of mechanisms could explain the association of Tac positivity with HTLV infection. HTLV infection may lead, directly or indirectly, to the activation of the gene encoding the production of the Tac antigen (the TCGF receptor). For example, a gene in the pX region of the virus may encode a protein involved in indirect Tac antigen expression. Alternatively, HTLV may enter the T cell through the Tac antigen, on T cells activated by another mechanism, and may clonally expand this cell population by transforming a cell bearing the Tac antigen. The observation that during replication and secretion of HTLV the virion becomes preferentially associated with the Tac receptor (42) is in accord with this view. Finally, HTLV may bear a protein that reacts with anti-Tac antibodies because it shares an antigenic determinant with Tac. However, against this view it should be noted that all HTLV-infected Tac-positive cells bind TCGF.

The fact that all ATL cell populations manifest the Tac antigen that is associated with the inducible receptor for TCGF may have implications for our understanding of one of the pathogenic factors associated with the leukemic transformation and with the rapid proliferation of these malignant T cells. As considered previously by Gallo and Wong-Staal (22), the infection of mature T cells with HTLV leads to the production of TCGF by some of these cell populations in culture and to the expression of the TCGF receptor in all cases. For those cases producing TCGF, there may be a self-stimulatory circuit in which the same cell produces and can respond to this growth factor for T cells. More recently, it has been shown that most HTLV-transformed cord T cell lines do not release detectable TCGF. It is thus possible that, in certain cases, HTLV infection leads to a bypassing of the TCGF-TCGF receptor system, and causes altered growth by mechanisms that have not been defined. Alternatively, HTLV may directly effect the receptor by changing its form, so that it behaves as if TCGF were bound to it, and, thus, it directly stimulates the growth of the malignant T cell.

Tsuda et al. (43) have shown that in contrast to the pattern with normal T cells, the TCGF receptor cannot be modulated from Tac-positive ATL cells. Studies in our own laboratory (44) have shown structural differences between the antigen detected by anti-Tac on certain ATL cells as compared with Tac receptors on activated normal T cells also supporting the view that ATL is associated with aberrant receptors for TCGF and that an abnormality in this receptor may play a role in growth of these leukemic cells.

References

1. Broder, S., R. L. Edelson, M. A. Lutzner, D. L. Nelson, R. P. MacDermott, M. E. Durm, C. K. Goldman, B. D. Meade, and T. A. Waldmann. 1976. The Sézary syndrome: a malignant proliferation of helper T cells. *J. Clin. Invest.* 58:1297-1306.
2. Broder, S., D. Poplack, J. Whang-Peng, M. Durm, C. K. Goldman, L. Muul, and T. A. Waldmann. 1978. Characterization of a suppressor cell leukemia: evidence for the requirement of an interaction of two T cells in the development of human suppressor effector cells. *N. Engl. J. Med.* 298:66-72.
3. Broder, S., and T. A. Waldmann. 1978. The suppressor cell network in cancer. *N. Engl. J. Med.* 299:1281-1284.
4. Reinherz, E. L., P. C. Kung, G. Goldstein, and S. F. Schlossman. 1979. Separation of functional subsets of human T cells by a monoclonal antibody. *Proc. Natl. Acad. Sci. USA.* 76:4061-4065.
5. Reinherz, E. L., P. C. Kung, G. Goldstein, and S. F. Schlossman. 1980. A monoclonal antibody reactive with the human cytotoxic/suppressor T cell subset previously defined by hetero-antiserum termed TH₂. *J. Immunol.* 124:1301-1307.
6. Reinherz, E. L., P. C. Kung, G. Goldstein, R. H. Levy, and S. F. Schlossman. 1980. Discrete stages of human intrathymic differentiation. Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. *Proc. Natl. Acad. Sci. USA.* 77:1588-1593.
7. Sutherland, R., D. Delia, C. Schneider, R. Newman, J. Kemstread, and M. Greaves. 1981. Ubiquitous cell-surface glycoprotein on tumor cell proliferation associated receptor for transferin. *Proc. Natl. Acad. Sci. USA.* 78:4515-4519.
8. Uchiyama, T., S. Broder, and T. A. Waldmann. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac⁺ cells. *J. Immunol.* 126:1393-1397.
9. Leonard, W. J., J. M. Depper, T. Uchiyama, K. A. Smith, T. A. Waldmann, and W. C. Greene. 1982. A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor. *Nature (Lond.).* 300:267-269.
10. Uchiyama, T., J. Yodoi, K. Sagawa, K. Takatsuki, and H. Uchino. 1977. Adult T-cell leukemia: clinical and hematological features of 16 cases. *Blood.* 50:481-492.
11. Hanaoka, M. 1982. Progress in adult T-cell leukemia research. *Acta Pathol. Jpn.* 32(Suppl.):171-185.
12. Blayney, D. W., W. A. Blattner, M. Robert-Guroff, E. S. Jaffe, R. I. Fisher, P. A. Bunn, Jr., M. G. Patton, H. R. Rarick, and R. C. Gallo. 1983. The human T-cell leukemia/lymphoma virus (HTLV) in the southeastern United States. *J. Am. Med. Assoc.* 250:1048-1052.
13. Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo. 1980. Detection and isolation of type-C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA.* 77:7415-7419.
14. Poiesz, B. J., F. W. Ruscetti, M. S. Reitz, V. S. Kalyanaraman, and R. C. Gallo. 1981. Isolation of a new type-C retrovirus (HTLV) in primary cultured cells of a patient with Sézary T-cell leukemia. *Nature (Lond.).* 294:268-271.
15. Gallo, R. C., D. Mann, S. Broder, F. W. Ruscetti, M. Maeda, V. S. Kalyanaraman, M. Robert-Guroff, and M. S. Reitz, Jr. 1982. Human T-cell leukemia-lymphoma virus (HTLV) is in T- but not B-lymphocytes from a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA.* 79:5680-5683.
16. Hinuma, Y., K. Nagata, M. Hanaoka, M. Nakai, T. Matsumoto, K. Kinoshita, S. Shirakawa, and I. Miyoshi. 1981. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc. Natl. Acad. Sci. USA.* 78:6476-6480.
17. Gallo, R. C., M. Popovic, F. W. Ruscetti, M. A. Wainberg, I. Royston, M. S. Reitz, Jr., S. Broder, and M. Robert-Guroff. 1981. Interaction of T-cell growth factor and a new retrovirus (HTLV) with human T cells. *Proc. 10th Annu. ICN-UCLA Sym. Molec. Cell. Biol.* F. Fox, editor. Alan R. Liss, Inc., New York. 231-246.
18. Popovic, M., P. S. Sarin, M. Robert-Guroff, V. S. Kalyanaraman, D. Mann, J. Minowada, and R. C. Gallo. 1983. Isolation and transmission of human retrovirus (human T-cell leukemia virus). *Science (Wash. DC).* 219:856-859.
19. Blattner, W. A., V. S. Kalyanaraman, M. Robert-Guroff, T. A. Listner, D. A. G. Galton, P. S. Sarin, M. H. Crawford, D. Catovsky, M. Greaves, and R. C. Gallo. 1982. The human type-C retrovirus, HTLV, in blacks from the Caribbean region, and relationship to adult T-cell leukemia/lymphoma. *Int. J. Cancer.* 30:257-264.
20. Kalyanaraman, V. S., M. G. Sarngadharan, P. A. Bunn, J. D. Minna, and R. C. Gallo. 1981. Antibodies in human sera reactive against an internal structural protein (p24) of human T-cell lymphoma virus. *Nature (Lond.).* 294:271-273.
21. Robert-Guroff, M., K. A. Fahey, M. Maeda, Y. Nakao, Y. Ito, and R. C. Gallo. 1982. Identification of HTLV-p19 specific natural human antibodies by competition with monoclonal antibody. *Virology.* 122:297-305.
22. Gallo, R. C., and F. Wong-Staal. 1982. Retroviruses as etiologic agents of some animal and human leukemias and lymphomas and as tools for elucidating the molecular mechanism of leukemogenesis. *Blood.* 60:545-557.
23. Brodsky, F. M., P. Parham, C. J. Barnstable, M. J. Crumpton, and W. F. Bodmer. 1979. Monoclonal antibodies for analysis of the HLA system. *Immunol. Rev.* 47:3-61.
24. Uchiyama, T., D. L. Nelson, T. A. Fleisher, and T. A. Waldmann. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature T cells. II. Expression of Tac antigen on activated cytotoxic T cells, suppressor cells and on one of two types of helper T cells. *J. Immunol.* 126:1398-1403.
25. Leonard, W. J., J. M. Depper, R. J. Robb, T. A. Waldmann, and W. C. Greene. 1983. Characterization of the human receptor for T cell growth factor. *Proc. Natl. Acad. Sci. USA.* 80:6957-6961.
26. Matheison, B. J., S. O. Sharrow, P. S. Campbell, and R. Asofsky. 1979. LyT differentiated thymocytes sub-population detected by flow microfluorometry. *Nature (Lond.).* 277:478-480.
27. Waldmann, T. A., S. Broder, R. M. Blaese, M. Durm, M. Black-

man, and W. Strober. 1976. Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinemia. *Lancet*. II:609-613.

28. Waldmann, T. A., and S. Broder. 1982. Polyclonal activators in the study of the regulation of immunoglobulin synthesis in the human system. In *Advances in Immunology*. H. Kunkel and F. J. Dixon, editors. Academic Press, Inc., New York. 32:1-63.

29. Keightley, R. G., M. D. Cooper, and A. R. Lawton. 1976. T cell dependence of B-cell differentiation induced by pokeweed mitogen. *J. Immunol.* 117:1538-1544.

30. Terhorst, C., A. VanAgthoven, K. LeClair, P. Snow, E. Reinherz, and S. F. Schlossman. 1981. Biochemical studies in the human thymocyte cell surface antigens T6, T9 and T10. *Cell*. 23:771-780.

31. Berger, C. L., D. Warburton, J. Raafat, P. Logerfo, and R. L. Edelson. 1979. Cutaneous T-cell lymphoma: neoplasm of T cells with helper activity. *Blood*. 53:642-651.

32. Kansu, E., and S. P. Hauptman. 1979. Suppressor cell population in Sézary syndrome. *Clin. Immunol. Immunopathol.* 12:341-350.

33. Hopper, J. E., and J. M. Haren. 1980. Studies on a Sézary lymphocyte population with T-suppressor activity. Suppression of immunoglobulin synthesis of normal peripheral blood lymphocytes. *Clin. Immunol. Immunopathol.* 17:43-54.

34. Uchiyama, T., K. Sagawa, K. Takatsuki, and H. Uchino. 1978. Effect of adult T-cell leukemia cells on pokeweed mitogen induced normal B-cell differentiation. *Clin. Immunol. Immunopathol.* 10:24-34.

35. Fleisher, T. A., W. C. Greene, T. Uchiyama, C. K. Goldman, D. L. Nelson, R. M. Blaese, and T. A. Waldmann. 1981. Characterization of a soluble suppressor of immunoglobulin production from a long-term T-cell line. *J. Exp. Med.* 154:156-167.

36. Biddison, W. E., P. E. Rao, M. A. Talle, G. Goldstein, and S. Shaw. 1982. Possible involvement of the OKT4 molecule in T cell recognition of class II HLA antigens: evidence from studies of cytotoxic T lymphocytes specific for SB antigens. *J. Exp. Med.* 156:1065-1076.

37. Krensky, A. M., C. S. Reiss, J. W. Mier, J. L. Strominger, and S. J. Burakoff. 1982. Long-term human cytolytic T cell lines allospecific for HLA-DR6 antigen are OKT4⁺. *Proc. Natl. Acad. Sci. USA*. 79:2365-2369.

38. Meuer, S. C., R. E. Hussey, J. C. Hodgdon, T. Hercend, S. F. Schlossman, and E. L. Reinherz. 1982. Surface structures involved in target recognition by human cytotoxic T lymphocytes. *Science (Wash. DC)*. 218:471-473.

39. Thomas, Y., L. Rogozinski, O. H. Irigoyen, S. M. Friedman, P. G. Kung, G. Goldstein, and L. Chess. 1981. Functional analysis of human T cell subsets defined by monoclonal antibodies. IV. Induction of suppressor cells within the OKT4⁺ population. *J. Exp. Med.* 154:459-467.

40. Morgan, D. A., F. W. Roscetti, and R. C. Gallo. 1976. Selective *in vitro* growth of T-lymphocytes from normal human bone marrows. *Science (Wash. DC)*. 193:1007-1008.

41. Blattner, W. A., D. W. Blayney, M. Robert-Guroff, M. G. Sarnagadharan, V. S. Kalyanaraman, P. S. Sarin, E. S. Jaffe, and R. C. Gallo. 1983. Epidemiology of human T-cell leukemia/lymphoma virus. *J. Infect. Dis.* 147:406-416.

42. Lando, Z., P. Sarin, M. Megson, W. C. Greene, T. A. Waldmann, R. Gallo, and S. Broder. 1983. Association of human T-cell leukemia/lymphoma virus (HTLV) with the Tac antigen marker for the human T-cell growth factor receptor. *Nature (Lond.)*. 305:733-736.

43. Tsudo, M., T. Uchiyama, K. Takatsuki, H. Uchino, and J. Yodoi. 1983. Modulation of Tac antigen on activated human T cells by anti-Tac monoclonal antibody. *J. Immunol.* 129:592-595.

44. Leonard, W. J., J. M. Depper, J. S. Roth, S. Rudikoff, T. A. Waldmann, and W. C. Greene. 1983. Aberrant T cell growth factor (TCGF) receptors on human T cell leukemia virus (HTLV) infected leukemia cells. *Clin. Res.* 31:348A.